Short Communication

A novel *MLPH variant in dogs with coat colour dilution*

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Summary

Coat colour dilution may be the result of altered melanosome transport in melanocytes. Loss-of-function variants in the melanophilin gene (*MLPH*) cause a recessively inherited form of coat colour dilution in many mammalian and avian species including the dog. *MLPH* corresponds to the *D* locus in many domestic animals and recessive alleles at this locus are frequently denoted with *d*. In this study we investigated dilute coloured Chow Chows that could not be explained by their genotype at the previously known MLPH:c.-22G>A variant. Whole genome sequencing of such a dilute Chow Chow revealed another variant in the *MLPH* gene, MLPH:c.705G>C. We propose to designate the corresponding mutant alleles at these two variants d^1 and d^2. We performed an association study in a cohort of 15 dilute and 28 non-dilute Chow Chows. The dilute dogs were all either compound heterozygous d^1/d^2 or homozygous d^2/d^2, while the non-dilute dogs carried at least one wildtype allele *D*. The d^2 allele did not occur in 417 dogs from diverse other breeds. However, when we genotyped a Sloughi family, in which a dilute coloured puppy had been born out of non-dilute parents, we again observed perfect co-segregation of the newly discovered d^2 allele with coat colour dilution. Finally, we identified a blue Thai Ridgeback, with the d^1/d^2 genotype. Thus, our data identify the MLPH:c.705G>C as a variant explaining a second canine dilution allele. Although relatively rare overall, this d^2 allele is segregating in at least three dog breeds, Chow Chows, Sloughis, and Thai Ridgebacks.

Keywords: Whole genome sequencing, melanocyte, melanosome, pigmentation, Canis lupus familiaris

Coat colour dilution refers to a specific pigmentation phenotype that is found in many mammalian and avian species. This phenotype may be caused by a defect in melanosome transport (Barral & Seabra, 2004). Variants in the three genes *RAB27A*, *MYO5A* and *MLPH*, which are indispensable for this process, have been found in humans with Griscelli syndrome types I to III (Pastural et al. 1997; Anikster et al. 2002; Ménasché et al. 2003; Ménasché et al. 2006). Variants in *RAB27A* and *MYO5A* typically have severe pleiotropic effects and lead to syndromic phenotypes, while *MLPH* variants mostly have a more restricted effect on coat colour only. *MLPH* variants are known in many spontaneous mammalian and avian mutants with a dilute coloured phenotype (Matesic et al. 2001; Ishida et al. 2006; Vaez et al. 2008; Bed'hom et al 2012; Cirera et al. 2013; Lehner et al. 2013; Fontanesi et al. 2014; Li et al. 2016). In dogs, a recessive non-coding variant at the last nucleotide of exon 1 in the *MLPH* gene (c.-22G>A) is associated with the dilution phenotype in many breeds and used for genetic testing (Philipp et al. 2005; Drögemüller et al. 2007).

Chow Chow breeders recently were confronted with unexpected genetic testing results as phenotypically dilute dogs were noticed that were not homozygous for the mutant A-allele at MLPH:c.-22G>A (Figure 1). The aim of the present study therefore was to identify the genetic basis of these dilute coloured dogs.

We analysed a cohort of 15 dilute Chow Chows with discordant genetic testing results and 28 non-dilute Chow Chows as controls. Pedigree records were consistent with a monogenic autosomal recessive inheritance of the dilute coat colour phenotype. We initially genotyped all dogs for the known MLPH:c.-22G>A variant. For ease of reading we designate the mutant allele at this variant d^1. None of the 15 dilute Chows was homozygous d^1/d^1, 6 were heterozygous, and 9 were homozygous wildtype at this position. We then sequenced the whole genome of a female Chow Chow with blue coat colour (diluted black) that was homozygous *wt/wt* at the MLPH:c.-22G>A variant. We prepared a PCR-free library, collected 192,197,978 read-pairs of 2 x 150 bp on an illumina HiSeq 3000 instrument and mapped the reads to the CanFam 3.1 Boxer reference genome yielding a ~23x coverage. The sequence data were submitted to the European Nucleotide Archive (project accession PRJEB16012, sample

accession SAMEA104091566). SNVs and short indels were called with respect to the reference genome using GATK (McKenna et al. 2010). We filtered for variants that were present in a homozygous alternate state in the dilute Chow Chow and heterozygous or homozygous reference in 190 non-dilute control dogs of different breeds and three wolves (Table S1). Among these private variants was a single nucleotide variant at the last nucleotide of exon 7 in the *MLPH* gene. The formally correct variant designation is NM_001103219.2:c.705G>C or Chr25:g.48,150,787G>C (CanFam3.1 assembly). The variant changes a codon, p.(Gln235His). It seems possible that the mutant allele affects splicing as the wildtype G-allele corresponds to the consensus of the mammalian 5'-splice site found in ~80% of all mammalian exon/intron junctions, whereas the mutant C-allele is found in only ~4% of comparable mammalian exon/intron junctions (Sheth et al. 2006). As we had no suitable RNA samples, we could not experimentally analyse the effect of the variant on splicing. We propose to designate the mutant allele at this variant as d^2. We genotyped all available Chow Chows by Sanger sequencing and found a perfect association with the dilution phenotype when considering also compound heterozygosity with the d^1 variant (Table 1, Figure S1).

While the vast majority of our control dogs with genome sequence information were homozygous wildtype for the newly discovered MLPH:c.705G>C variant, we noticed two Sloughis that were heterozygous. These two Sloughis were parents of a litter that included a puppy with dilute coat colour and blue eyes (Figure 1E). We genotyped the entire Sloughi family and found again perfect co-segregation of the d^2 allele with the phenotype: Only the blue puppy was homozygous d^2/d^2 while the non-dilute littermates were either *wt/wt* or wt/d^2 (Table 1). Finally, we identified a blue Thai Ridgeback, with the d^1/d^2 genotype (Figure 1F). The d^2 allele was not present in 417 additional control dogs of 56 different breeds (Table S2).

Given the extensive knowledge on melanophilin function, we think that these data strongly suggest that the newly discovered MLPH:c.705G>C variant causes coat colour dilution in dogs and represents another loss-of-function allele similar to MLPH:c.-22G>A. This finding of allelic

heterogeneity in canine coat colour dilution needs to be considered when performing genetic testing.

Dilute coloured dogs, which are homozygous d^1/d^1, are predisposed for colour dilution alopecia (CDA), a disease characterized by hair loss and chronic inflammation of the skin (Miller, 1990; Kim et al. 2005; von Bomhard et al. 2006; Welle et al. 2009). It is currently not fully clear, which additional genetic and/or environmental risk factors are involved in CDA. However, CDA seems to be a dog-specific phenomenon. Other known *MLPH* mutant animals with dilute coloured phenotypes, such as e.g. cats, rabbits, or mice apparently have no pathological alterations of their hair or skin. While the coat colour phenotype in d^1/d^1, d^1/d^2 or d^2/d^2 dogs is identical or at least very similar, it is at this time not possible to make a reliable prediction whether these 3 genotypes show any differences with respect to their risk for CDA. The blue Thai Ridgeback in our study with the d^1/d^2 genotype showed mild signs of CDA. Thus, the association of the *d2* allele with CDA should be carefully investigated before breeding recommendations are given.

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Conflicts of interest

Alexandra Kehl is an employee of Laboklin, a company that offers genetic testing as a commercial service.

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Table 1 Association of *MLPH* genotypes with coat colour dilution. The mutant alleles at MLPH:c.-22G>A and MLPH:c.705G>C are designated d^1 and d^2, respectively.

MLPH genotype	*wt/wt*	*wt/d*^{1}	*wt/d*^{2}	*d*^{1}*/d*^{1}	*d*^{1}*/d*^{2}	*d*^{2}*/d*^{2}
Dilute Chow Chows (cases)	-	-	-	-	6	9
Non-dilute Chow Chows (controls)	8	4	16	-	-	-
Dilute Sloughi (case)[1]	-	-	-	-	-	1
Non-dilute Sloughis (controls)[1]	1	-	6	-	-	-
Dilute Thai Ridgeback (case)	-	-	-	-	1	-

[1]The eight Sloughis came from a family consisting of both non-affected parents, 5 non-affected littermates and one dilute puppy.

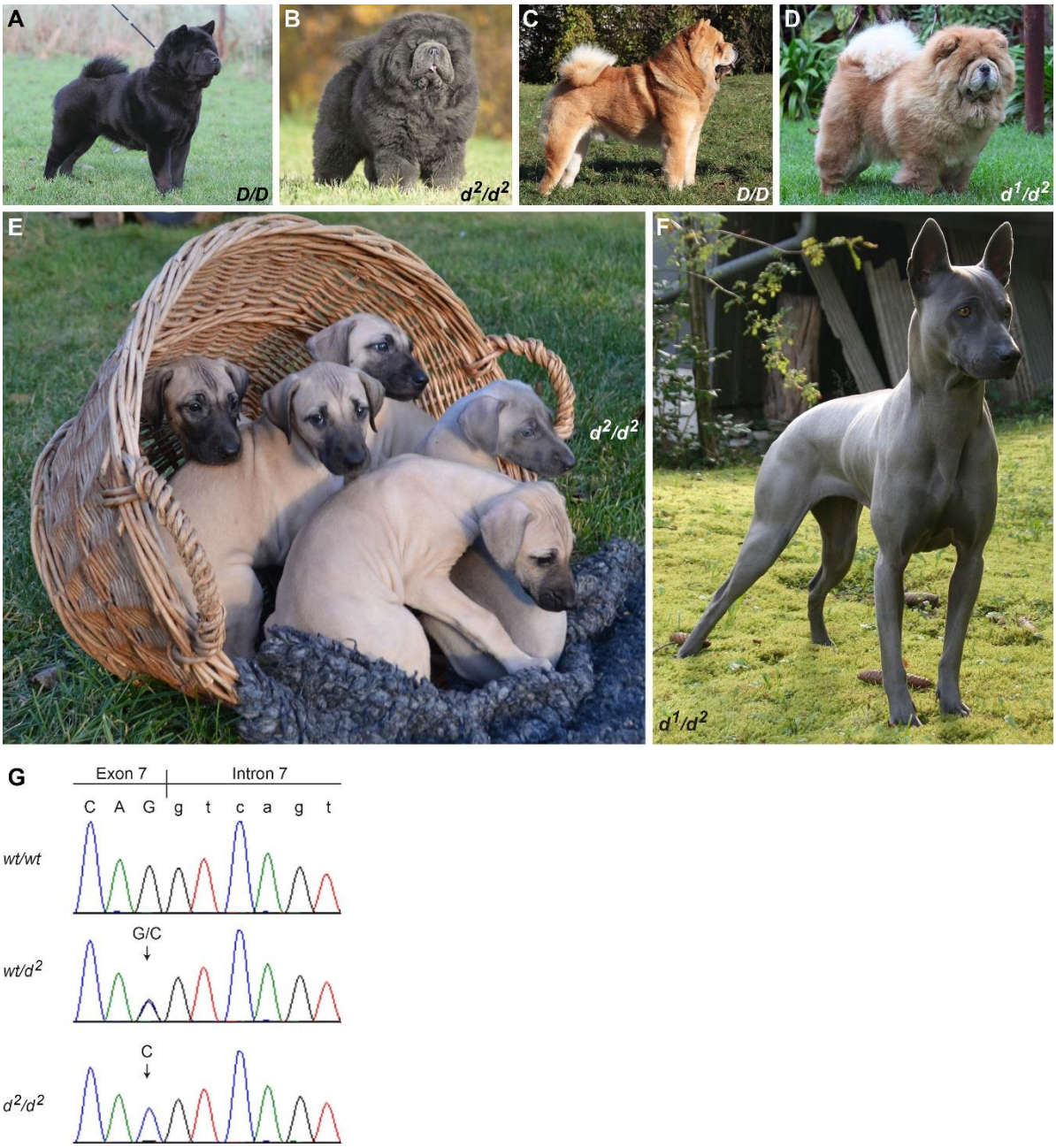

Figure 1 Coat colour dilution phenotype and the MLPH:c.705G>C variant. Coat colour dilution leads to phenotypically lighter coat colours. Dilute dogs with a black base colour are termed blue, dilute red dogs are termed fawn or cinnamon. (A-D) Representative images from black, blue, red, and fawn Chow Chows are shown. (E) Sloughi puppies at 6 weeks of age. The rightmost puppy is dilute. In this dog the black pigment of the mask is much lighter and the dog has blue eyes (photo courtesy of Ingela Näslund). The dilute coat colour phenotype in this dog became less apparent with aging. (F) Blue Thai Ridgeback. (G) Sanger sequencing electropherograms of dogs with the three genotypes at the MLPH:c.705G>C variant. We propose to designate the mutant C-allele at this variant as d^2.

Supplementary Material

Figure S1. *MLPH* haplotypes confirm compound heterozygosity.

Table S1. Accession numbers of 191 dog/wolf genome sequences.

Table S2. Genotypes of 417 dogs from different breeds